ing point in admixture with authentic IVb. The recovered weight was 1.0 g. The sodium hydroxide solution filtrate (above) was first acidified with dilute hydrochloric acid and then neutralized by addition of sodium bicarbonate. The precipitated product was filtered, dried and recrystallized from benzene, giving 0.1 g., m.p. 140.5–142.5°. This material gave no depression in melting point in admixture with authentic VII.

1-Methylamino-2-chloro-3-ketotetrafluorocyclopentene-1 (VII) also never was prepared by deliberate acid or alkaline hydrolysis of IIb in such a way as to determine optimum conditions. It was encountered as a by-product from attempts to alkylate IIb with methyl iodide using sodium ethoxide in ethanol to generate the anion of IIb and as the minor product from acid hydrolysis of VIb. A recommended procedure for its preparation would be to subject IIb to aqueous acid hydrolysis until VII crystallized from the reaction mixture in satisfactory yield; VII was soluble in dilute alkaline solution and sparingly soluble in cold water or dilute acid solution. It crystallized well from benzene solution, m.p. 141–142°.

Anal. Calcd. for $C_6H_4ONF_4Cl$: C, 33.10; H, 1.84; N, 6.44; Cl, 16.32; F, 34.94. Found: C, 33.52; H, 2.38; N, 6.73; Cl, 16.97; F, 35.3.

1-Azido-2-chlorohexafluorocyclopentene-1.—A solution of 47.5 g. (0.73 mole) of sodium azide in 600 ml. of warm dimethyl sulfoxide was placed in a steam-jacketed dropping funnel (to prevent crystallization) and was added to a stirred suspension of 170 g. (0.695 mole) of 1,2-dichlorohexafluorocyclopentene-1 in 700 ml. of dimethyl sulfoxide over a period of 3.5 hours. The reaction temperature stayed between 28–36° without external cooling. Immediately after completion of addition a test for azide ion with ferric chloride was negative. (A second molar equivalent of sodium azide readily may be consumed in such a reaction mixture. The product, considerably more orange colored than the monoazido compound described above, detonated with great violence upon the only occasion when its distillation was attempted. An undistilled sample of this material was found to have an impact sensitivity of one inch/1 kg. weight *vs.* cyclotrimethylene trinitramine = 12 inches.) The solution was poured into two liters of ice and water (to compensate for the considerable heat of solution of dimethyl sulfoxide). The lower layer was separated, washed again with water, giving 146 g. of crude product. This was dried over Drierite, keeping the product in the refrigerator. The product was distilled at 46 mm. pressure. After a small forerun, b.p. 37–48°, nearly the entire remainder was collected in one fraction, b.p. 48–50°, $n^{20}D$ 1.4135, wt. 122.6 g., 48.8% yield. This material could be kept indefinitely without noticeable decomposition when stored in the refrigerator. Samples stored at room temperature developed pressure within a day or two. A redistillation attempted at 75° head temperature was interrupted by excessive rise in pressure. Distillations at 45–50° never have given trouble.

Anal. Calcd. for $C_5N_3F_6Cl$: C, 23.86; N, 16.70; Cl, 14.11. Found: C, 24.13; N, 15.24; Cl, 13.63.

1,3,3-Trimethoxy-2-chlorotetrafluorocyclopentene-1.—A solution of 13.8 g. (0.6 mole) of sodium metal in 300 ml. of absolute methanol was prepared in a 1-liter three-necked flask equipped with a stirrer, dropping funnel and reflux condenser protected with a drying tube. Forty-nine grams (0.2 mole) of 1,2-dichlorohexafluorocyclopentene-1 was added to the stirred sodium methoxide solution at a rate sufficient to cause brisk refluxing. Precipitation of sodium halides was observed at once. The reaction mixture was heated for an additional 3 hours and allowed to stand overnight. Precipitated solids were removed by filtration and the filtrate was mixed with 1.5 liters of water. The mixture was extracted with two 200-ml. portions of ether. The combined ether extracts were washed twice with equal volumes of water and dried with magnesium sulfate. Solvent was evaporated and the residue distilled at 6.4 mm. giving: (1) 0.8 g., b.p. 78–80°; (2) 36.0 g., b.p. 80–81° (68% yield); and 3.4 g. of residue. Fraction 2 was redistilled at 5.8 mm. giving a small forerun and 33.7 g., b.p. 80–81°, $n^{20}D$ 1.4225. The infrared spectrum of this material showed contamination by a small amount of carbonyl-containing compound (see Discussion). It would appear probable that such contamination might be reduced by avoiding the aqueous extraction step in the work-up procedure.

Acknowledgment.—The writer wishes to express appreciation and indebtedness to Dr. Keith S. McCallum and members of the Analytical Staff for microanalyses, spectroscopic measurements and much help in the evaluation and interpretation of these data.

HUNTSVILLE, ALA.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, NORTH DAKOTA AGRICULTURAL COLLEGE]

The Glucosides of Flaxseed. II. Linocaffein[1]

BY HAROLD J. KLOSTERMAN AND ROBERT Z. MUGGLI

RECEIVED SEPTEMBER 25, 1958

A second crystalline glucoside obtained by the alkaline methanolysis of the complex polymer present in flaxseed hulls is shown to be methyl 4-β-D-glucopyranosylcaffeate (I). The structure of I, which is designated *linocaffein*, has been established by degradation and synthesis.

An earlier paper[2] described the isolation of a brown, amorphous, gum-like substance from the hull of flaxseed. Alkaline methanolysis of this gum was shown to give rise to the methyl esters of β-hydroxy-β-methylglutaric acid,[3] 4-glucosyloxycinnamic acid (linocinnamarin),[2] together with a mixture of unknown glucosides and phenolic constituents. One of these glucosidic components is shown herein to be methyl 4-β-D-glucopyranosylcaffeate (I) and it has been named linocaffein.

Caffeic acid has been isolated from a wide variety of plant materials. It is found either free or combined in an unknown manner. Various investigators have suggested that caffeic acid also occurs in nature as the glucoside, although this had not been previously substantiated.

The yellow glucosidic mixture which remained after the isolation of the β-hydroxy-β-methylglutaric acid[3] was examined for phenolic glucosides by partition chromatography using an ammoniacal butanol–ethanol–water mixture as the partitioning liquid. The dried chromatogram was sprayed with the bis-diazotized benzidine reagent used by Linstedt[4] in the detection of phenolic compounds to give a series of brown colored spots and one deep pink

(1) Presented before the Division of Carbohydrate Chemistry at the 132nd National American Chemical Society Meeting, New York, N. Y., September, 1957. Published by permission of the Director, North Dakota Agricultural Experiment Station.

(2) H. J. Klosterman, F. Smith and C. O. Clagett, THIS JOURNAL, **77**, 420 (1955).

(3) H. J. Klosterman and F. Smith, *ibid.*, **76**, 1229 (1954).

(4) G. Linstedt, *Acta Chem. Scand.*, **4**, 448 (1950).

colored spot which also showed a positive test for glycosides.[5]

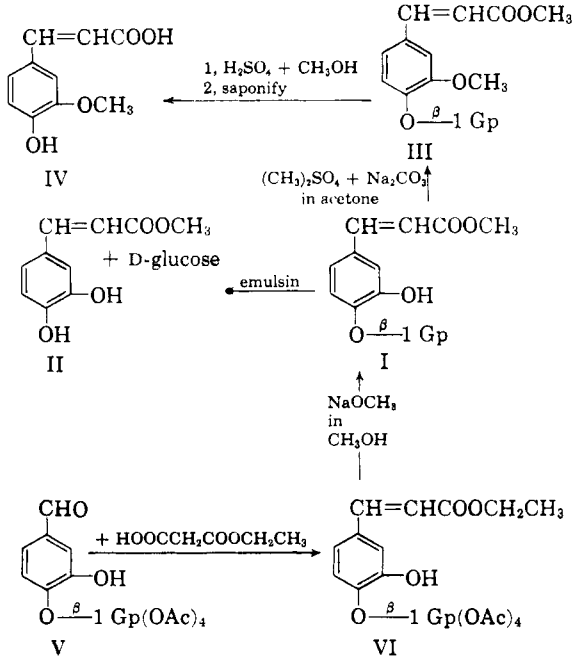
The glucoside I was isolated from the glucosidic mixture[3] remaining after the isolation of β-hydroxy-β-methylglutaric acid by chromatography on a cellulose column followed by displacement from an anion exchange resin. The successful isolation of I was facilitated by the fact that it gave a deep pink color with diazotized benzidine. This color was very intense and did not fade over a period of several months.

The glucoside I showed a strong selective absorption in the ultraviolet, the absorption curve resembling those obtained for hydroxylated cinnamic acids.[6] The infrared spectrum indicated the presence of ester and ethylenic groups. Since hydrogenation with palladium catalyst caused a profound change in the ultraviolet absorption, two of the four bands disappearing, it was deduced that the ethylenic group was conjugated with an aromatic system. The specific extinction coefficient was greatly reduced at the same time.

The glucoside I contained a single methoxyl group.

Although acid hydrolysis of I gave rise to D-glucose and a resinous material, enzymic cleavage with β-glucosidase (emulsin) gave rise to D-glucose and methyl 3,4-dihydroxycinnamate (II) (methyl caffeate). The latter also was obtained from I by the action of methanol containing a catalytic amount of sulfuric acid.

Methylation of the aglucon II yielded methyl 3,4-dimethoxycinnamate which like II existed in several characteristic crystalline forms.



Methylation of the glucoside I yielded a product III which no longer exhibited phenolic properties. Treatment of III with acidic methanol afforded an oil which upon saponification yielded a crystalline acid IV which was identified as ferulic acid, 3-methoxy-4-hydroxycinnamic acid. Since the methoxyl group at C_3 had been introduced during methanolysis and since the hydroxyl group at C_4 resulted from the cleavage of the glucose residue, it was apparent that glucoside I must be the methyl ester of 4-D-glucosylcaffeic acid. The high levo rotation of I ($[\alpha]^{25}_D$ −87.3°) and the observation that the D-glucose residue is cleaved by β-D-glucosidase clearly indicated that I is a β-D-glucoside. Linocaffein (I) therefore was designated tentatively as methyl 4-β-D-glucopyranosylcaffeate.

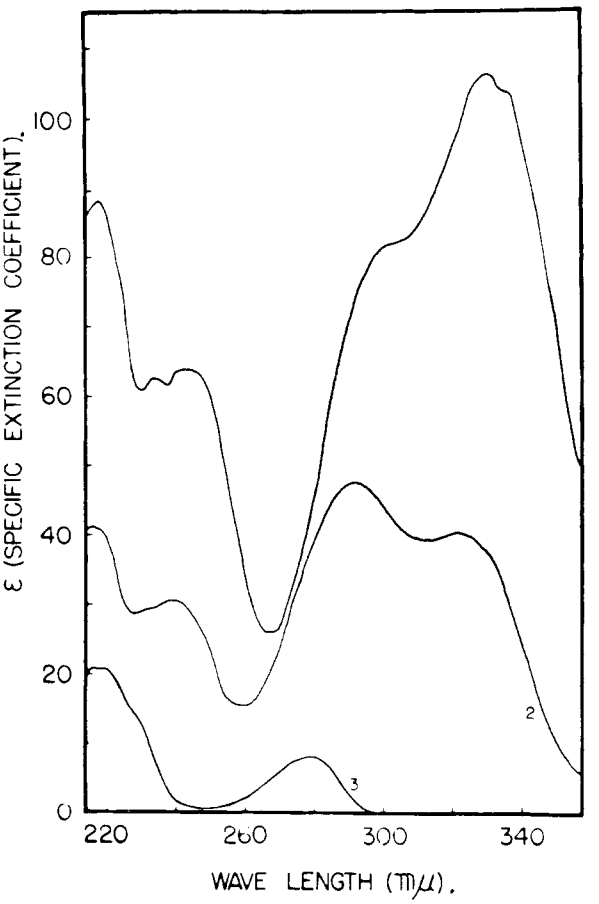


Fig. 1.—Absorption spectrum in 95% ethanol: 1, aglucon (methyl caffeate); 2, linocaffein; 3, hydrogenated linocaffein.

Final proof of the structure of I assigned to linocaffein was provided by the observation that the treatment of 3-hydroxy-4-tetra-O-acetyl-β-D-glucosyloxybenzaldehyde (V)[7] with ethyl hydrogen malonate gave VI which upon deacetylation by the Zemplèn method yielded methyl 4-β-D-glucopyranosylcaffeate.

The manner in which this new glucoside I, linocaffein, and the two previously identified compounds, β-hydroxy-β-methylglutaric acid and linocinnamarin, are combined in the complex is not known. Since all three substances are obtained as methyl esters when the complex is treated with sodium methoxide, it would appear that the components are joined to the complex by ester linkages.

(5) J. G. Buchanan, C. A. Dekker and A. G. Long, *J. Chem. Soc.*, 3162 (1950).

(6) H. Dannenberg, *Z. Naturforsch.*, **4b**, 336 (1949).

(7) B. Helferich and F. Vorsatz, *J. prakt. Chem.*, **145**, 270 (1936).

Experimental

The melting points were determined by use of a microscope equipped with a heated stage and are uncorrected.

Isolation of Linocaffein (I).—Four grams of the polymer from flaxseed[2] was stirred with 0.8 g. of sodium methoxide and 40 ml. of dry methanol for 15 hr. at room temperature.

The resulting slurry was centrifuged to remove a small insoluble fraction. The clear brown liquid was adjusted to *p*H 4 with sulfuric acid using methyl orange as an external indicator and the solution centrifuged to remove sodium sulfate.

The methanol was evaporated under reduced pressure, the resulting sirup dissolved in 10 ml. of a mixture of 1-butanol–ethanol–water (10:1:1) and fractionated by chromatography on a cellulose column with the same solvent mixture, the eluate being collected in 5-ml. fractions. By means of the diazotized benzidine reagent,[4] the fractions giving the deep pink color were located, combined and evaporated.

The light brown solid which resulted was dissolved in 15 ml. of 50% aqueous ethanol, and freed from cations by passing through a cation exchange resin (Amberlite IR-120). The phenolic glucoside I, linocaffein, was adsorbed on Duolite A4 anion resin and recovered from the anion resin by elution with 50 ml. of a solution of 2 *N* acetic acid in 50% aqueous ethanol. The solvent was evaporated under reduced pressure, water being added several times during the distillation to facilitate removal of the acetic acid. Recrystallization of the residue from methanol gave fine needles of linocaffein (I), m.p. 208°, $[\alpha]^{25}D$ −87.3° (*c* 1, methanol). The maximum yield of the glucoside was about 1% of the polymer. Linocaffein gave a positive hydroxamic acid test for esters and a positive test for phenols by the Folin reagent and the diazotized benzidine reagent[4]. The ultraviolet absorption spectrum of linocaffein (I) in 95% ethanol is shown in Fig. 1.

Anal. Calcd. for $C_{16}H_{20}O_9$: C, 53.9; H, 5.6; OCH_3, 8.7; mol. wt., 356. Found: C, 53.6; H, 5.6; OCH_3, 9.0; mol. wt. (Rast, camphor), 360.

Hydrogenation of the Glucoside I.—A solution of the glucoside I (25 mg.) in 25 ml. of methanol was hydrogenated at room temperature at 2 atm. pressure using 0.1 g. 10% palladium-on-charcoal as the catalyst. The absorption curve after 2 hr. is shown in Fig. 1. The great reduction in the specific extinction coefficients indicated that hydrogenation had occurred with probable removal of some conjugated unsaturation. The small amount of material available did not permit a quantitative determination of hydrogen uptake.

The methanol solution from the hydrogenation reaction was filtered and evaporated under reduced pressure. The sirup that remained was treated with 5% sulfuric acid for 6 hr. at 100°. Evaporation of an ethereal extract of the hydrolysis mixture gave 3,4-dihydroxyphenylpropionic acid (10 mg.), m.p. 135° (found: OMe, nil), lit.[8] m.p. 140°.

Hydrolysis of the Glucoside I.—Linocaffein (I) (50 mg.) was dissolved with heating in a mixture of 0.2 *M* acetate buffer (*p*H 5.0) (6 ml.) and methanol (0.5 ml.). After cooling to room temperature, emulsin (1 mg., Nutritional Biochemicals) was added and the mixture incubated at 37° for 12 hr. Extraction with ether gave crystalline methyl caffeate (II) (25 mg.). Recrystallization from benzene yielded broad needles, m.p. 140–142°, the melt resolidifying as cubic crystals which melted at 157–158°. Rapid cooling of molten II gave fine needles, m.p. 125°. Recrystallization of II from benzene–chloroform–methanol (5:5:1) yielded short fine needles, m.p. 158–159°. Freudenberg and Heel[9] reported that methyl caffeate occurs in two forms, m.p. 152–153°, 158–160°.

Anal. Calcd. for $C_{10}H_{10}O_4$: C, 61.9; H, 5.2; OCH_3, 16.0. Found: C, 62.0; H, 5.6; OCH_3, 15.2.

The aqueous layer from the enzymatic degradation of I which reduced Fehling solution was evaporated to dryness. Treatment of the residue with *p*-nitroaniline gave N-(*p*-nitrophenyl)-D-glucosylamine, m.p. and mixed m.p. 184°.

Hydrolysis of linocaffein in boiling 5% sulfuric acid gave rise to a tar and D-glucose which was identified in the above manner.

Methylation of Compound II (Aglucon) with Methyl Sulfate.—A methylation mixture consisting of 80 mg. of compound II (aglucon), 1 ml. of methyl sulfate, 10 ml. of acetone and 2.5 g. of anhydrous potassium carbonate was refluxed for 2.5 hr. At this time the Folin reagent gave a negative test for phenols. About 5 ml. of water was added and the solution heated to destroy the excess methyl sulfate. The acetone was removed by heating on a steam-bath while passing air over the surface of the solution. As the acetone evaporated a white solid separated. This was extracted with ether to separate it from the inorganic salts. Evaporation of the ethereal extract gave methyl 3,4-dimethoxycinnamate (95 mg.).

When recrystallized from dilute methanol, plates formed which melted at 61–62°. On further heating prismatic crystals formed at 64° and melted at 67–68°. Tiemann and Will[10] reported that methyl 3,4-dimethoxycinnamate existed in a prismatic form which melted at 64°. The ultraviolet absorption spectrum of the methylated aglucon was determined in 95% ethanol and found to be identical with known methyl 3,4-dimethoxycinnamate.

Methylation of the Glucoside I with Methyl Sulfate.—Sixty-five milligrams of the glucoside I was dissolved in 10 ml. of methanol and 0.4 ml. of methyl sulfate; 4 ml. of 1 *N* sodium methoxide in methanol was added in 0.2-ml. increments as needed to maintain alkalinity in the reaction mixture. After 6 hours the solution showed only a slight positive test with Folin reagent and bis-diazotized benzidine indicating that the phenolic function had been largely methylated. The methylated product III was not isolated.

Methanolysis of the Methylated Glucoside III and Isolation of Ferulic Acid (IV).—The methanolic solution of the methylated glucoside III from the preceding experiment was treated with 0.33 ml. of sulfuric acid (sp. gr. 1.95) and refluxed 5 hr.

The solution was neutralized to methyl orange (external indicator) with solid sodium bicarbonate and the solvents were removed under reduced pressure. The solids were dissolved in water and extracted with ether; 33 mg. of ether-soluble material was recovered from the 65 mg. of the glucoside I originally used. The oily phenolic ester so obtained failed to crystallize. A paper chromatogram of this compound using butanol–benzene–water (1:9:10) as a solvent indicated 2 spots when sprayed with Folin reagent, an intense spot, R_f 0.95, and a faint spot, R_f 0.75. The faint spot probably was due to a dihydroxy compound resulting from incomplete methylation of the aglucon.

The oil was dissolved in the above chromatogramming solvent and passed over a small cellulose column to separate the two materials. The fast running fraction was collected and the solvent removed. Again an oil was recovered which resisted all attempts to cause it to crystallize.

The oil (*ca.* 30 mg.) was refluxed with 1 ml. of 1 *N* sodium hydroxide for 30 min. An ether extraction was made to remove any unreacted compounds. The solution was made slightly acidic with hydrochloric acid. The free acid IV was extracted from the acidified solution with ether. Evaporation of the ether yielded 3-methoxy-4-hydroxycinnamic acid (ferulic acid), m.p. and mixed m.p. 169–170°, after recrystallization from benzene–methanol (10:1); lit.[11] m.p. 170°.

Synthesis of Linocaffein.—The procedure of Helferich, *et al.*,[7] was used to prepare 3-hydroxy-4-(tetra-O-acetyl-β-D-glucosyloxy)-benzaldehyde (V). A solution of 5.6 g. of V in 6 ml. of ethyl hydrogen malonate and 6 ml. of pyridine was heated at 55° for 24 hr. The mixture was cooled and poured into ice-water causing the separation of a brown oil which gradually crystallized. Recrystallization from ethanol yielded 2 g. of the acetylated glucoside VI which melted at 98–100°.

Deacetylation and simultaneous conversion to the desired methyl ester was achieved by treating the acetylated glucoside VI (2 g.) with sodium methoxide (1 g.) in anhydrous methanol (40 ml.). The solution was refluxed 15 min., cooled and freed of cations by passage through a column of a cation exchange resin. Evaporation of the solvent yielded white needles, 1.0 g., m.p. 186–196°. Repeated recrystallizations failed to give a sharp melting product. Chromatography of the product on paper using a mixture of 1-butanol–ethanol–diethylamine–water (150:50:50:2) as the irri-

(8) E. O. von Lipmann, *Ber.*, **26**, 3063 (1893).

(9) K. Freudenberg and W. Heel, *Chem. Ber.*, **86**, 190 (1953).

(10) F. Tiemann and W. Will, *Ber.*, **14**, 959 (1881).

(11) F. B. Power and F. Tutin, *J. Chem. Soc.*, **91**, 893 (1904).

gating liquid disclosed two components with R_f 0.74 and 0.65. The slower moving component was the major constituent and migrated at the same rate as linocaffein (I). The location of the components was determined by spraying the chromatogram with bis-diazotized benzidine.[4]

The crude product from the deacetylation reaction was fractionated on a cellulose column using the above irrigating liquid. Evaporation of the fractions which contained the slower moving component gave linocaffein which crystallized from hot water as needles, $[\alpha]^{24}D$ −88° (*c* 1, methanol), m.p. 206–208° alone or in admixture with the linocaffein (I) obtained from the flax hulls.

The faster moving component was not characterized but was probably an isomer of linocaffein with the glucose residue attached to the ring in the 3-position.

FARGO, N. DAK.

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DEPARTMENT OF THE SCHERING CORPORATION]

A New Class of Potent Anti-inflammatory Agents; Synthesis of 9α,11β-Dihalocorticosteroids

BY C. H. ROBINSON, L. FINCKENOR, EUGENE P. OLIVETO AND DAVID GOULD

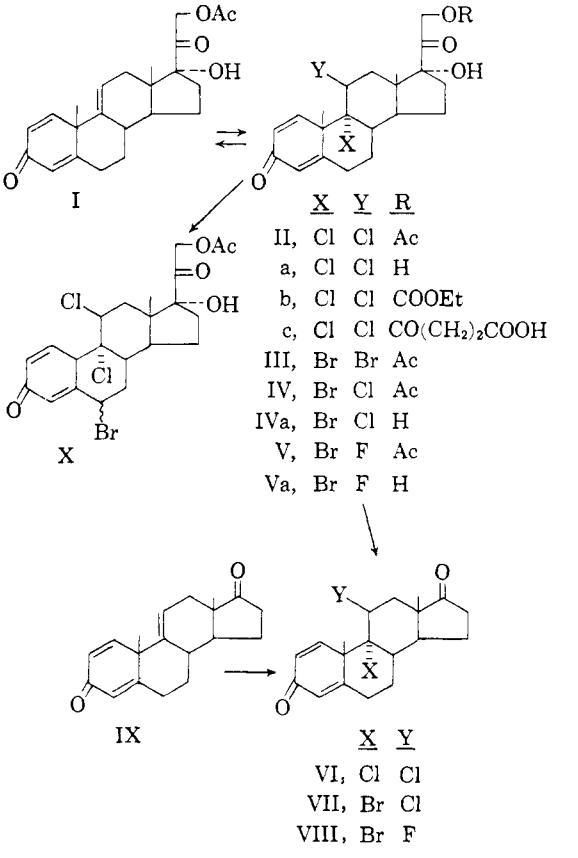
RECEIVED SEPTEMBER 8, 1958

A number of 9α,11β-dihalo steroids have been prepared by the addition of halogens and mixed halogens to the 9(11)-double bond of some 1,4,9(11)-steroidal trienes. Certain of the dihalides are powerful anti-inflammatory agents, as measured by the granuloma pouch test.

The enhancement of anti-inflammatory and glucocorticoid activity associated with the insertion of a chlorine or fluorine atom at C-9 in 11-oxygenated corticoids is well established.[1-3] This increased activity shown by compounds containing *trans*-9,11-chlorohydrin and fluorohydrin systems led us to speculate that *trans*-9,11-dihalocorticoids might also show anti-inflammatory activity, and the ready addition of hypobromous acid to 9(11)-olefins[1,3,4] encouraged us to study the addition of halogens and mixed halogens to 1,4,9(11)-pregnatriene-17α,21-diol-3,20-dione-21-acetate[3] (I).

When a solution of the triene I and lithium chloride[5] in acetic acid was treated with 1.1 moles of chlorine (preferably derived from N-chlorosuccinimide and hydrogen chloride, although solutions of chlorine in organic solvents can also be successfully employed) a halogen-containing compound was isolated in 58% yield. Elemental analysis indicated that this compound had resulted from the addition of 1 mole of chlorine to the triene I. We formulate this product as 9α,11β-dichloro-1,4-pregnadiene-17α,21-diol-3,20-dione-21-acetate (II) on the following grounds.

The infrared spectrum of II showed that both the 1,4-diene-3-one system and the cortical side chain were intact, and the ultraviolet absorption (λ_{max} 237 mμ, ε 15,000) and positive tetrazolium reaction of II provided supporting evidence. Chromous chloride in acetone at room temperature (sodium iodide in acetone provoked no reaction) smoothly converted II into the triene I, demonstrating that no skeletal rearrangement had occurred in the formation of II.



That both chlorine atoms were located in the nucleus became apparent from the following reaction sequence. Hydrolysis of II using methanolic perchloric acid[4] gave the 21-alcohol IIa which was degraded with sodium bismuthate[6] to a dichloroandrostadienedione. The latter compound also could be prepared by the addition of chlorine to 1,4,9(11)-androstatriene-3,17-dione (IX) and

(1) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953); **76**, 1455 (1954).

(2) R. F. Hirschmann, R. Miller, R. E. Beyler, L. H. Sarett and M. Tishler, *ibid.*, **77**, 3166 (1955).

(3) J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman and F. M. Singer, *ibid.*, **77**, 4181 (1955).

(4) J. Fried and E. F. Sabo, *ibid.*, **79**, 1130 (1957).

(5) Without added chloride ions, mixtures of 9α,11β-dichloride and 9α-chloro-11β-acetate resulted. When lithium acetate was substituted for lithium chloride the 9α-chloro-11β-acetate was isolated as the major product. The literature contains several examples of the addition of the elements of acyl hypohalite to double bonds, and pertinent references are given the accompanying paper (C. H. Robinson, L. Finckenor, M. Kirtley, D. H. Gould and E. P. Oliveto) which describes the preparation of a series of 9α-halo-11β-acyloxy corticoids.

(6) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).